PREDOMINANCE OF TYPE I COLLAGEN AT THE SURFACE OF AVIAN ARTICULAR CARTILAGE

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Received 31 October 1977

1. Introduction

The collagen in hyaline cartilages of mammals, including articular cartilage of the major joints, appears to be all of molecular type II [1–3]. In contrast, fibrocartilage, such as that of the knee meniscus, contains mainly type I collagen [3], and intervertebral disc, a specialized fibrocartilage, contains types I and II distributed in opposing concentration gradients in a highly specific manner [4,5].

Articular cartilage of the chicken was shown to differ from that of the mammal by containing a significant proportion of type I collagen in addition to type II [6,7]. Since it has been suggested that a switch in synthesis from type II to type I collagen in human articular cartilage may be a crucial factor in the pathogenesis of osteoarthrosis [8–10], the distribution and role of type I in normal avain articular cartilage are important to evaluate.

In the present study, changes with age and with depth from the articular surface in the proportions of types I and II collagens in chicken knee cartilage have been assessed. The total content of type I increased after hatching. becoming the major collagen component at maturity. At all ages type I was essentially the only collagen type at the articular surface and it gradually interchanged with type II with increasing depth. The content of hexosamines, and hence of proteoglycans, increased with depth apparently in unison with the rising type II content. Cartilages from other major joints of the chicken, and from other birds, all contained the two types of collagen, suggesting that this composite architecture is a phylogenetic trait of avian articular cartilage, distinguishing it from that of the mammal.

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2. Materials and methods

2.1. *Tissue sampling*

White Rock chickens and embroys were supplied by a wholesale farm. Domestic ducks and geese and wild Mallard ducks and Canadian geese were purchased from a retail butcher. Embryonic chick cartilage was carefully sampled from the femur and tibiotarsus of day 17 embryos taking for analysis only the terminal 1 mm at the articular ends. Whole metatarsals of day 5–6 embryos were also analysed. These consisted entirely of cartilage and showed no ossification centers by histology. Femoral knee cartilage was sampled from chickens of various ages, pooling tissue from several birds of the same age group. About two-thirds full thickness was taken.

Blocks of cartilage with reasonably flat articular surfaces (2 × 2 mm) were cut from femoral condyles of week 10 and week 15 chickens and sectioned on the freezing microtome into serial slices (100 μm) starting at the articular surface. Several adjacent blocks were cut from each condyle so that different chemical analyses could be performed on comparable slices of tissue. In addition the surface 3 μm of articular cartilage was sampled and pooled from 50 blocks of cartilage that were individually embedded in methacrylate and cut on a microtome using a glass knife.

2.2. *Analysis*

The proportions of types I and II collagens in the large pooled samples of chicken articular cartilage were measured by analysis of CNBr-peptides after digestion with CNBr in 70% formic acid, as previously described for porcine and human cartilages [3,5,11]. The two peptides, α_1(I)CB2 and α_1(II)CB6, were isolated as a combined fraction by column chromatography on phosphocellulose (fig.1). Although differing slightly in amino acid composition from their mammalian counterparts (Eyre, unpublished), their relative amounts could also be quantified by amino acid analysis of the mixture, knowing that each peptide contains certain amino acids absent from the other [3,5 11].

Collagen CNBr-peptides in digests of single slices of cartilage were fractionated by electrophoresis in slabs of SDS–polyacrylamide (10%) in Tris–borate buffer [12]. Other series of slices were analysed for (i) hydroxylysine and hydroxyproline after hydrolysis in 6 M HCl for 24 h at 108°C; (ii) hydroxylysine glycosides after hydrolysis in 2 M NaOH for 24 h at 108°C [3]; or (iii) glucosamine and galactosamine after hydrolysis in 6 M HCl for 3 h at 100°C [13]. Suitable elution conditions for accurately measuring the above amino acids and amino sugars were established on the Beckman 121M amino acid analyser. To measure absolute hexosamine or hydroxyproline contents, slices of cartilage (about 200 μg dry wt) were dried to constant weight and weighed to the nearest μg on a Cahn electrobalance before hydrolysis.

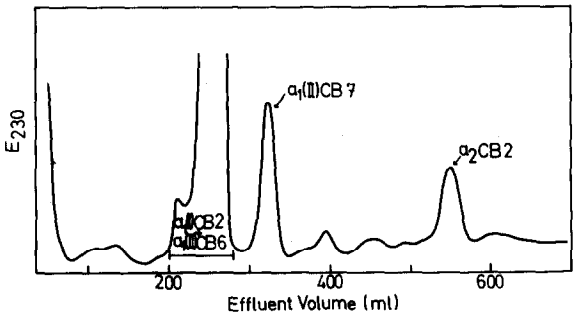

Fig.1. Chromatography on phosphocellulose of CNBr-peptides of collagen from week 10 chicken articular cartilage. Peptides from 200 mg of digest were eluted from a column (1.5 × 10 cm) at 43°C with a linear gradient of 0–0.3 M NaCl in 800 ml 1 mM sodium formate, pH 3.6. The partially resolved peptides, α_1(I)CB2 and α_1(II)CB6, were pooled, as indicated, for analysis.

3. Results

3.1. *Chicken cartilage*

The ration of type I to type II collagen in pooled samples of knee cartilage rose dramatically as the chicken matured. Thus, only type II collagen could be detected by electrophoresis of collagen CNBr-peptides prepared from the whole day 5–6 metatarsals and from the articular ends of the epiphyseal cartilage of day 17 embryos (not shown). From week 10 chickens three separate preparations of pooled cartilage contained type I collagen at 35 (±5)% of the total collagen, the remainder being type II when measured by quantitative analysis of CNBr-peptides. By week 20 and week 30, type I collagen measured 80 (±5)% and 75 (±5)%, respectively, of the total collagen in the pooled samples.

When serial slices (100 μm thick) of femoral cartilage from surface to deep regions were analyzed, type I collagen predominated at the surface and type II below about the first 500 μm. In fact, the propor-

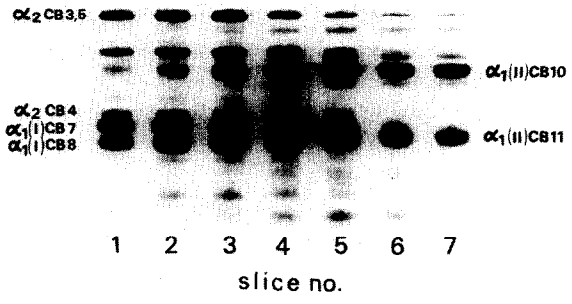

Fig.2. Electrophoresis of collagen CNBr-peptides prepared from serial slices of chicken articular cartilage. The slices (each 100 μm thick and about 2 × 2 mm) were taken from femoral knee cartilage of a week 15 chicken, progressing with depth from the articular surface (surface = slice no.1). A sample (20–40 μg) of the complete CNBr digest of each slice (100–200 μg dry wt) was run in a slab (1.5 mm thick) of polyacrylamide (10%) using Tris–borate buffers containing sodium dodecyl sulphate, and stained with Coomassie blue [12]. Peptide bands were identified by comparison with analyses of control collagens. Those identified on the left of the gel are the major peptides derived from type I, and those on the right from type II collagen.

Table 1
Changes with depth from the surface in the collagen composition of chicken articular cartilage[a]

Depth ($\mu m \times 10^2$)	Hydroxylysine content (Residues/100 hydroxyproline residues)		Degree of glycosylation of hydroxylysine (%)		(glcgalHyl/galHyl)[b]	
	10 wk	15 wk	10 wk	15 wk	15 wk	15 wk
0–1	13.3	13.7	18	17	1.3	1.2
1–2	12.6	–	26	21	1.3	1.0
2–3	15.6	15.8	29	24	1.2	1.1
3–4	18.0	18.3	34	27	1.5	1.2
4–5	20.8	20.2	36	30	1.5	1.2
5–6	21.8	20.6	34	39	1.4	1.5
6–7	23.8	22.4	51	44	2.0	1.6
7–8	–	23.6	54	44	2.1	1.6
8–9	23.4	23.8	56	41	2.1	1.4
9–10	22.2	24.8	61	51	2.2	1.7
Whole articular cartilage from day 17 embryos	22.5		57		5.7	
Surface 3 μm from week 15 chickens	10.2		–		–	

[a] Cartilage from femoral condyles of the knee of 10 week and 15 week old chickens was analysed
[b] Molar ratio of glucosylgalactosylhydroxylysine to galactosylhydroxylysine

tions of the two collagen types gradually interchanged, with a smooth transition from almost all type I in the surface 100 μm to almost all type II by several slices deep (fig.2). This was obvious at all ages, though type I collagen extended deeper and clearly accounted for more of the total collagen by week 20, in agreement with the quantitative analysis of pooled tissue at this age.

Type I collagen unusally contains less than 10 Hyl residues/100 Hyp with 20% glycosylated, and type II about 20 Hyl with 60–70% glycosylated. The steep increase in ratio of hydroxylysine to hydroxyproline (Hyl/100 Hyp), and in the percentage of hydroxylysine residues glycosylated (table 1), therefore confirmed the smooth change from mainly type I to mainly type II collagen through the surface layers of cartilage. The pooled surface slices of 3 μm thickness were also analysed for Hyl/100 Hyp content (table 1) and gave the lowest value of all, indicating that type I alone was present at the articular surface.

The rise in hexosamine content with depth (table 2) suggests a rise in proteoglycan content from about 5% dry wt in the surface 100 μm to about 30% dry wt at 1 mm deep. The rise in molar ratio of galactosamine/glucosamine with depth suggests either a gradual change in proteoglycan quality such as an increasing ratio of chondroitin sulphate to keratan sulphate, or an increasing ratio of proteoglycans to glycoprotein, or both. The combined proteoglycan and collagen contents do indicate that more dry weight is unaccounted for towards the surface, as was concluded previously for bovine cartilage [14].

3.2. *Other species*

Knee and wing joints of other domestic birds, and of actively flying birds form the wild, were also examined. All samples of cartilage from tibiotarsus and femur of the knee and from ulna and humerus of the elbow of chicken, turkey, Mallard duck, domestic duck, Canadian goose and domestic goose, contained a mixture of types I and II collagens when examined by electrophoresis of CNBr-peptides (not shown).

The tibial and ulnar cartilages of all species, including chicken, consistently revealed a higher ratio of type I/type II than did the femoral and humeral cartilages of the same joints, as assessed semiquantitatively by the electrophoresis of peptides. The histological appearance of the avian cartilages also varied among

Table 2
Changes with depth from the surface in the collagen and hexosamine content of chicken articular cartilage[a]

Depth ($\mu m \times 10^2$)	Collagen content[b] (% dry wt)	Hexosamine content (% dry wt)	Galactosamine glucosamine
0–1	57	1.3	3.6
1–2	–	2.0	5.8
2–3	55	2.7	6.1
3–4	62	3.1	6.6
4–5	56	3.7	6.8
5–6	57	4.5	7.4
6–7	53	5.0	7.6
7–8	48	5.4	7.7
8–9	46	6.0	7.4
9–10	43	5.9	7.2

[a] Adjacent blocks of cartilage from a femoral condyle of the knee of a week 10 chicken were analysed
[b] Calculated from weight of hydroxyproline recovered × 7.6

and within joints, from overtly fibrocartilaginous, particularly at the surface of the tibial and ulnar cartilages, to a hyaline texture in humeral and femoral cartilages virtually indistinguishable from that of mammalian joints. The variation in histological appearance was enhanced, however, by lengthy demineralisation of joints, which probably brought out the fibrous texture of the articular cartilage by leaching out proteoglycans. Nevertheless, no matter how hyaline or fibrous the articular cartilage appeared, both types of collagen were always prominent chemically.

4. Discussion

The present work suggests that avian articular cartilages in general may contrast with mammalian by containing a large proportion of type I collagen which is concentrated at the articular surface. Thus, during growth and maturation of articular cartilage in the chicken, type II collagen is gradually replaced by type I so that by week 20 type I becomes the major collagen species of the mature tissue, and essentially the only collagen species at the articular surface. Type II, however, still the predominant collagen of the deep articular cartilage, probably including the basal calcified zone. The ratio of type I/type II measured in pooled samples of cartilage will obviously depend on the average depth of sampling, so the slight difference observed between the pooled samples of week 20 and week 30 tissues is not significant.

In the developing chick embryo, type I collagen has been detected immunohistologically at the articular surface of the epiphyseal growth cartilage, persisting until at least 3 days after hatching [15]. The presence of type I was explained as the vestiges of an epiphyseal perichondrium at the articular surface that was still differentiating into true articular cartilage [15]. The present findings show, however, that by maturity, rather than being replaced by type II collagen, type I collagen has become the predominant collagen of the tissue.

Generally, type II collagen appears to be associated with a high concentration of proteoglycans in hyaline cartilages, whereas type I predominates in more pliable fibrocartilages that contain less proteoglycans. Avian articular cartilage seems to have incorporated both features in a specialized composite matrix. It is clearly meaningless to attempt to categorize the tissue rigidly as hyaline or fibrous cartilage because a complete spectrum of textures can be found depending on the site and depth of surface examined. The important conclusion is that although most mammalian articular cartilages are typically hyaline and contain type II as their major collagen, these properties are not fundamental to the function of articular cartilage as the bearing surface of the diarthrodial joint.

The possibility that type I collagen of avian cartilage is associated with proteglycans of a different molecular type to those of the type II-enriched deeper regions of tissue should be examined. Two fractions of proteoglycans have been identified in developing cartilage of the chick embryo [16], but their relationship with collagen types is unknown.

Acknowledgements

We thank Ms Linda Lewi for expert technical assistance. This project was supported in part by a grant from the National Institutes of Health (AM 15671). D.R.E. is a fellow of The Medical Foundation, Boston, MA (Nelson E. Weeks Fund).

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